Evaluation of Indian transgenic *Bt* cotton and non *Bt* cotton against *Spodoptera litura* Fab. (Noctuidae: Lepidoptera) fourth and fifth instar larvae

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ABSTRACT

The laboratory evaluation of certain released Indian transgenic *Bt* cotton hybrids (RCH 2 *Bt* , RCH 515 *Bt* , RCH 596 *Bt*, RCH 530 *Bt*, RCH 134 *Bt* and RCH 533 *Bt*) for their efficacy on *Spodoptera litura* (Fab.) fourth and fifth instar larvae was done and comparison was made with non *Bt* cotton (non *Bt* cotton *viz*., Non *Bt* bunny and RCH 2 non *Bt* cotton) plant parts, young green bolls, top fully opened young leaves, middle leaves and squares. Studies were conducted at Insectary, Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore during 2009 -2010 to study the effect of transgenic *Bt* cotton hybrids *viz.*, RCH 2 *Bt* young green bolls which showed highest per cent mortality (15.86 %), top fully opened young leaves (14.50%), squares (13.50%) and middle leaves (13.50 %) and observed at 168 hrs after the treatments (HAT) followed by green bolls, top fully opened young leaves, squares and middle leaves of RCH 515 *Bt* that recorded 12.00, 10.50, 10.00 and 9.50 per cent mortality respectively after 168 HAT when compared to green bolls, top fully opened leaves, squares and middle leaves of RCH 2 non *Bt* cotton which recorded no mortality at all after 168 HAT. All four plant parts of *Bt* and non *Bt* cotton (green bolls, top fully opened young leaves, squares and middle leaves) recorded no mortality in fifth instar larvae of *S. litura* after at 168 HAT.

Keywords: Mortality, *Spodoptera litura*, Transgenic *Bt* cotton, toxicity

INTRODUCTION

In India transgenic cotton plant was developed by incorporating the bacterium, *Bacillus thuringiensis* Berliner (*Bt*). *Bt* is a gram positive, spore forming bacterium that produces a variety of insecticidal *Cry*stal proteins (ICP's) toxic to lepidopteran, dipteran and coleopteran larvae in the field and laboratory (Hofte and Whiteley, 1989; Benedict *et al.*, 1992; Crickmore *et al*., 1998 and Schnepf *et al*., 1998; Gore *et al.,* 2001; Jeyakumar *et al.*, 2008; Arshad *et al.,* 2009; Govindan *et al.*, 2009; 2010a, b; Arshad and Suhail, 2011; Selvi *et al.*, 2012*).* *Bt* transgenic cotton acreages have increased rapidly in recent years around the world (James, 2004; Kranthi *et al.,* 2011) because *Bt* toxins are environmentally safe and reduce the use of broad spectrum insecticides (Lambert and Peferoen, 1992; Akin *et al.,* 2011). However, growing *Bt* cotton successively raises the potential for resistance adaptation of the target insects to the toxins (McGaughey and Whalon, 1992; Sudarani and Rath, 2011; *Selvi et al*., 2012).

Insect resistant Indian transgenic *Bt* cotton produced insecticidal *Cry*stal protein from the soil bacterium *B. thuringiensis* and it was released for commercial use during 1996 in USA. In India, bollgard *Bt* gene of Monsanto was introduced into the Indian cotton hybrids developed by MAHYCO (Maharashtra hybrid seed company limited Mumbai). In India transgenic cotton accounted for an estimated 90 per cent of the total 11.1 million ha in 2010-11 (Kranthi, 2011). *Bt* cotton is cultivated in 35 per cent irrigated and 65 per cent rainfed land. In India, among the states, Maharashtra tops in the area under *Bt* cotton followed by Andhra Pradesh, Gujarat and Tamil Nadu (Anonymous, 2008; Kranthi *et al.*, 2011). *Bt* cotton has increased the yield by 64 per cent and pesticide usage has been reduced by 25 per cent.

The Indian Market Research Bureau International (IMRBI) survey indicates that bollgard crop of 2005-2006 helped Indian farmers to earn an additional income of Rs. 2,100 crores. The net profit increase for bollgard farmers is Rs. 6,727 per acre over conventional cotton farmers (Anonymous, 2007). Leaf worm, *Spodoptera litura* Fab. (Noctuidae: Lepidoptera) is one of the most destructive pests of cotton, which feeds on foliage and sometimes young green bolls. It is a secondary pest of cotton (Allen *et al.*, 2000). However, transgenic *Bt* cotton with *Cry*1Ac proved not to be effective against *Spodoptera* spp. (Ponsard *et al*., 2002; Hofs *et al*., 2004; Yu *et al*., 2004; Arshad and Suhail, 2011; Selvi *et al.,* 2012; Lalitha *et al.,* 2012). It has been found that *S. litura* has a greater potential to survive in the presence of *Bt* toxins when compared to other bollworms. In the present study, four different plant parts of *Bt* and non *Bt* cotton hybrids were tested for their effect on fourth and fifth instar larvae of *S. litura*.

MATERIALS AND METHODS

Laboratory experiments were carried out during 2009–2010 to study the effect of transgenic *Bt* cotton and non *Bt* cotton plant parts against fourth and fifth instar larvae of *S. litura*

Mass culturing of *Spodoptera litura*

The field collected egg masses of *S. litura* were used to initiate the mass culturing under laboratory conditions. The egg masses were kept in the egg cage. After emergence, first instar larvae were transferred to the castor leaves. The newly emerged larvae settled on the leaves and the leaves were taken and kept in the conical flask containing water. Five day old larvae were transferred to plastic buckets with castor leaves kept in conical flask containing water at the rate of 25 larvae/bucket. The leaves were changed and the faecal pellets removed from the container every 24 hrs. The grown up larvae were allowed to pupate in soil. Moths were collected on emergence and released in oviposition cage for egg laying. The required larvae for the different treatments were taken from the culture.

Host plants

Six Bollgard II (*Cry* 1 Ac + *Cry* 2 Ab genes) *Bt* cotton and two non *Bt* cotton hybrids were o*Bt*ained from Rasi Seeds (P) Ltd, Auttur, Salem (District), Tamil Nadu. Seeds were grown in mud pots in greenhouse with one plant in each pot. Thirty five days after sowing, the plants had approximately 15 nodes. The green bolls, top fully expanded young leaves, squares and middle leaves were used for the experiments.

Bt cotton and non *Bt* cotton on *S. litura* larvae

Plant parts of *viz.*, green young bolls, top young fully opened leaves, squares and middle leaves of *Bt* and non *Bt* cotton were used to feed the fourth and fifth instar larvae of *S. litura*. Insect bioassay studies were conducted. Plant parts of cotton were placed on moistened filter paper kept in Petri dish and 30 larvae were released into each Petri dish. To avoid drying of plant parts, the filter paper was moistened at regular intervals. The leaves, bolls and squares were changed at 24 hrs interval and the fecal pellets and dead larvae were removed from the Petri dish every 24 hrs. The experiment was conducted under laboratory condition (28 ± 1^{0}C and 80 ± 5% RH). Mortality was recorded at 24 hrs intervals for seven days. Three replications were maintained for each treatment.

Statistical analysis

The methods of Gomez and Gomez (1984) were followed in scrutinizing the data from various experiments. Square root and angular transformations were adopted for the data in numbers and percentage respectively (Abbott, 1925). Means in simple CRD analysis were separated by Duncan's multiple range test (Duncan, 1951).

RESULTS AND DISCUSSION

Green young bolls

The results on toxicity of green young bolls of *Bt* and non *Bt* cotton hybrids (Table 1) revealed that significant difference in the mortality level of fourth instar larvae on green young bolls could be observed from the bioassay results. In all seven hybrids no mortality was observed up to 96, 120,144 hrs after treatments (HAT). At 168 hrs maximum mortality was observed in green young bolls of RCH 2 *Bt* (15.86%), which was on par with RCH 515 *Bt* (12.00%), RCH 596 *Bt* (10.37%), RCH 530 *Bt* (10.33%), RCH 134 *Bt* (9.66%), RCH 533 *Bt* (9.33%) and *Bt* bunny (9.33%). No morality was observed in green young bolls of non *Bt* cotton hybrids at 168 HAT.

Table 1. Per cent mortality of fourth instar larva of *Spodoptera litura* on different transgenic *Bt* and non *Bt* cotton

Treatments	**% *Mortality (in hrs)**																			
	Green young bolls					**Top younger fully opened leaves**					**Squares**					**Middle leaves**				
	96	**120**	**144**	**168**	**Mean**	**96**	**120**	**144**	**168**	**Mean**	**96**	**120**	**144**	**168**	**Mean**	**96**	**120**	**144**	**168**	**Mean**
***Bt* bunny**	0.00	0.00	0.00	9.33^{ab}	2.33	0.00	0.00	0.00	9.34^{ab}	2.33	0.00	0.00	0.00	9.35^{ab}	2.34	0.00	0.00	0.00	9.25^{ab}	2.31
Non *Bt* bunny	0.00	0.00	0.00	0.00^{c}	0.00	0.00	0.00	0.00	0.00^{c}	0.00	0.00	0.00	0.00	0.00^{b}	0.00	0.00	0.00	0.00	0.00^{c}	0.00
RCH 2 *Bt* BG II	0.00	0.00	0.00	15.86^{a}	3.60	0.00	0.00	0.00	14.50^{a}	3.62	0.00	0.00	0.00	13.50^{a}	3.38	0.00	0.00	0.00	13.50^{a}	3.38
RCH 2 non *Bt*	0.00	0.00	0.00	0.00^{c}	0.00	0.00	0.00	0.00	0.00^{c}	0.00	0.00	0.00	0.00	0.00^{b}	0.00	0.00	0.00	0.00	0.00^{c}	0.00
RCH 515 *Bt* BG II	0.00	0.00	0.00	12.00^{ab}	3.00	0.00	0.00	0.00	10.50^{ab}	2.62	0.00	0.00	0.00	10.00^{a}	2.50	0.00	0.00	0.00	9.50^{ab}	2.38
RCH 596 *Bt* BG II	0.00	0.00	0.00	10.37^{ab}	2.59	0.00	0.00	0.00	10.36^{ab}	2.59	0.00	0.00	0.00	8.00^{ab}	2.00	0.00	0.00	0.00	8.35^{ab}	2.09
RCH 530 *Bt* BG II	0.00	0.00	0.00	10.33^{ab}	2.58	0.00	0.00	0.00	9.32^{ab}	2.33	0.00	0.00	0.00	8.00^{ab}	2.00	0.00	0.00	0.00	7.16^{ab}	1.79
RCH 134 *Bt* BG II	0.00	0.00	0.00	9.66^{ab}	2.41	0.00	0.00	0.00	9.65^{ab}	2.41	0.00	0.00	0.00	8.50^{ab}	2.13	0.00	0.00	0.00	8.33^{ab}	2.08
RCH 533 *Bt* BG II	0.00	0.00	0.00	9.33^{ab}	2.33	0.00	0.00	0.00	9.33^{ab}	2.33	0.00	0.00	0.00	9.50^{ab}	2.38	0.00	0.00	0.00	8.33^{ab}	2.08

* Mean of three replications; BG II (*Cry* 1 Ac + *Cry* 2 Ab genes); In a column means followed by a common letter are not significantly different by DMRT (P=0.05)

The results are in line with Govindan *et al.*, 2010, who observed that second instar larvae of *S. litura* top fully opened young leaves, squares, middle leaves and young green bolls of RCH 515 *Bt* recorded 46.67, 38.34, 37.67 and 30.00 per cent mortality respectively after at 168 HAT. Moreover, Selvi *et al.* (2012) stated that NCEH 14 *Bt* and NCEH 3R *Bt* recorded 29.48 and 52.08 % respectively in first instar larvae of *S. litura*. The *Helicoverpa armigera* (Hubner) (Men *et al.*, 2005; Arshad *et al.*, 2009) and *H. zea* (Gore *et al.*, 2001) in which larvae avoided food treated with *Bt* insecticides or transgenic plants. Zhang *et al.* (2004) found that *H. armigera* neonates showed lower consumption and higher mortality on transgenic cotton leaves compared to non transgenic cotton leaves.

Top younger fully opened leaves

The results on toxicity of top younger fully opened leaves of *Bt* and non *Bt* cotton hybrids (Table 1) revealed that all the seven hybrids and two non *Bt* showed no mortality up to 144 HAT. At 168 HAT, hybrid RCH 2 *Bt* showed 14.50 per cent mortality which was followed by RCH 515 *Bt* (10.50%); other three hybrids *viz.*, RCH 596 *Bt* (10.36%), RCH 530 *Bt*, RCH 134 *Bt* and RCH 533 *Bt* were at par in their efficacy. In the case of non *Bt* cotton, no mortality was observed at 168 HAT. Similar results were also reported by Govindan *et al.* (2009), who observed RCH 2 *Bt* young green bolls (42.68%), top fully opened young leaves (37.67%), squares (35.00%) and middle leaves (25.83%) mortality in second instar larvae in *S. litura* at 168 hrs after the treatments. In the field experiment the Widestrike™ *Bt* Cotton, Expressing *Cry*1Ac and *Cry*1F Proteins, population of *S. litura* was lower in WS hybrids, (< 0.02 larva/ plant) and higher in non-WS hybrids (0.15 and 0.72 larva per plant (Moudgal *et al.*, 2011). Benedict *et al.*, (1992) had observed the feeding deterrent effect of *Bt* cotton leaves against *Heliothis virsecens.* Li *et al.* (2006) found that the percentage of feeding damage by *Trichoplusia ni* larvae on *Bt* cotton was only 15.67%, while 84.47% damage was recorded in non *Bt* cotton. Abro *et al.* (2004) reported 13.3 to 53.3% mortality of *Spodoptera* spp. on different *Bt* cotton (*Cry*1Ac) varieties.

Squares

The toxicity level of squares of *Bt* and non *Bt* cotton hybrids (Table 1) indicated that all hybrids showed no mortality up to 144 HAT. Maximum mortality was found in RCH 2 *Bt* (13.50 %) followed by RCH 515 *Bt* (10.00 %) which was on par with (RCH 533 *Bt* (9.50%), RCH 134 *Bt* (8.50%), RCH 596 *Bt* (8.00%), RCH 530 *Bt* (8.00%) and *Bt* bunny (9.35 %), whereas no mortality was recorded in non *Bt* cotton (Non *Bt* bunny and RCH 2 non *Bt*) at 168 hrs after treatment. Low larval mortality was observed in *Spodoptera* spp in later instar, feeding on *Bt* cotton (*Cry*1Ac) leaves (Ashfaq and Young, 1999; Henneberry *et al*., 2001; Abro *et al*., 2004; Sudaharani and Rath, 2011; Selvi *et al.*, 2012). The results reported by Murugan *et al.* (2003) indicated that first instar larvae of *H. armigera* when reared on the squares of transgenic cultivars showed highest mortality at 72 hrs. The results reported by Lalitha *et al.* (2012) who indicated that the larval mortality was found are increases significantly with increase in *Bt* concentrations and also Prasad and Rao (2008) and Prasad *et al.* 2009 reported that the *Bt* hybrids were found highly effective against *H. armigera* with a very low larval population of pink bollworm.

Middle leaves

The results on toxicity of middle leaves of *Bt* and non *Bt* cotton hybrids (Table 1) showed no mortality up to 144 HAT. The highest mortality was noticed in hybrid RCH 2 *Bt* (13.50 %), which was on par with RCH 515 *Bt* (9.50%), *Bt* bunny (9.25%), RCH 596 *Bt* (8.35%), RCH 134 *Bt* (8.33%) and RCH 533 *Bt* (8.33%) at 168 hrs after treatment. No mortality was noticed in non *Bt* cottons, non *Bt* bunny and RCH 2 non *Bt* These findings are in conformity with the findings of Govindan *et al.* (2009) who reported that RCH 2 *Bt* young green bolls showed highest per cent mortality (54.31%), squares (43.88%), middle leaves (43.68 %) and top fully opened young leaves (40.00%) in second instar larvae of *S. litura* observed at 168 hrs after the treatments (HAT) and also Banna *et al.* (2012) reported that younger larvae are generally more susceptible than older larvae because of their peritrophic matrix bindings. Bagade *et al*. (2005) reported that transgenic *Bt* cotton was found effective against three bollworms (*H. armigera*, *Earias spp.* and *P.gossypiella*). Also

Luttrell *et al.* (1998) reported more tolerance in *S. frugiperda* against *Cry* 1 Ac than other bollworms.

_Bt_ cotton and non _Bt_ cotton on _S. litura_ fifth instar larvae

The toxicity level of all four plant parts viz., green bolls top, fully opened young leaves, squares and middle leaves of seven *Bt* hybrids and two non *Bt* cotton recorded no mortality in fifth instar larvae at 168 hr after treatment. The above was in accordance with the findings of Govindan *et al.* (2010), who reported that *Bt* toxin was not effective against later instar larvae of *S. litura* and with those of Selvi *et al.* (2012), who observed that NCS 207 *Bt* registered lower mortality than older larvae of *S. litura*. It was in agreement with the report of Arshad and Suhail (2011). *Cry*1Ac was not effective against beet armyworm, *S. exigua* in the laboratory experiments Chakroun *et al.* (2012). *Cry* toxin influences the toxicity to vary the species of *Spodoptera*. *S. exigua* was less susceptible than *S. frugiperda*. Hence, it can be concluded that all *Bt* cotton hybrids are slightly toxic to fourth insatr larvae of *S. litura* at 168 HAT, under laboratory conditions. The present study has identified the persistence of the *Bt* toxicity to *S. litura*.

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